

BD

PATENT SPECIFICATION

NO DRAWINGS

839,300



Date of Application and filing Complete Specification:
August 25, 1958.

No. 27188/58.

Application made in United States of America on
September 5, 1957.

(Patent of Addition to No. 757,934 dated January 18, 1954)

Complete Specification Published June 29, 1960.

Index at Acceptance: Class 81(1), B2(C:F:S:Z), B12A.

International Classification: A61k.

Adrenocorticotrophic hormone preparations and process for making same.

COMPLETE SPECIFICATION

We, ORGANON LABORATORIES LIMITED, a British Company, of Brettenham House, Lancaster Place, London, W.C.2, do hereby declare the invention, for which we pray that
5 a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:

This invention relates to new preparations
10 of the adrenocorticotrophic hormone, to be defined hereinafter with the commonly used abbreviation "ACTH", and is an improvement in or modification of the invention of Specification No. 757,934.

15 Specification No. 757,934 describes and claims aqueous suspensions of ACTH with enhanced and prolonged action and suitable for injection, which contain a complex of ACTH and one or more salts, hydroxides or
20 oxides of the metals zinc, nickel, cobalt, copper and iron, which complex is sparingly soluble at the pH of the tissue fluids.

In these suspensions use is mostly made of the metal zinc as this metal occurs in the
25 body fluids.

These suspensions of zinc-ACTH are of great practical value and are used on a large scale. They can be prepared by adding a
30 salt of zinc to an ACTH-solution followed by adjusting the pH of the preparation to the desired value.

The present invention is based on the observation that the preparations of Specification No. 757,934 can be improved by combining electrolytically prepared alpha-zinc
35 hydroxide or a basic zinc salt with ACTH in an aqueous medium. Said electrolytically prepared basic zinc salts have the same crystallographical characteristics as said
40 alpha-zinc hydroxide.

The products of the present invention offer improved advantages over the known products. Among these advantages are noted the smaller particle size of the instant pro-
45 ducts; their ease of suspension in suitable

(Price 3s. 6d.)

vehicles; their resistance to breakdown when subjected to alternate freezing and thawing; and their slower settling after re-suspension and the ease of manufacturing.

In conducting our novel process we may
50 form the desired complex in situ during the electrolytic preparation of the zinc compound; or, if desired, we may first prepare the zinc compound electrolytically and then add the ACTH to a suspension of the zinc
55 compound to form the complex. While, in general, the results obtained by either method are satisfactory and the particular method followed is optional, we presently prefer to form the desired complex in situ as the union
60 between the components appears more firm when the complex is formed in that manner.

It is noted that whichever method is employed in accordance with this invention the same conditions regarding electrolytic
65 formation of the zinc compound and proportions of ingredients apply. For purposes of the following general description of our present process we shall therefore only refer to the first of the alternative methods in
70 order to avoid repetition.

The process is conducted in a conventional electrolytic cell suitable for the production of zinc ions at the anode and hydroxyl ions at the cathode. Such a cell can have a zinc
75 anode and a platinum or other inert cathode, such as silver, gold and graphite, the electrolyte advantageously being an aqueous solution of sodium chloride or other pharmaceutically-acceptable electrolyte. A suitable
80 amount of ACTH is dissolved into the electrolyte. Other materials, such as preservatives and buffers, may be introduced into the electrolyte, if desired, it being understood that any such substance employed must
85 be compatible with the preparation and the human body in the concentrations employed and must not cause any irritating, toxic or other untoward effects.

A suitable electric current, e.g., from a 90

battery source, is passed through the cell. The amount of current flowing through the cell is measured and controlled by a milliammeter and rheostat, respectively, these being connected in series in the circuit.

The electrolysis generates alpha-zinc hydroxide which may contain a small amount of basic zinc salt, the nature of the salt being determined by the composition of the carrier electrolyte. When, for instance, chloride ions are present, a part of the zinc may be precipitated as the basic zinc chloride which has the same crystallographical characteristics as the alpha-zinc hydroxide. Any of the formed zinc compounds carries the ACTH down with it as a flocculent precipitate. The electrolysis is continued until the desired amount of zinc precipitate has been formed, this amount being from 0.2 to 3.0 mg. of elemental zinc per 20-80 units, preferably 40 units, of ACTH. The term "units" as used herein in reference to biological activity, refers to U.S. Pharmacopoeia units as determined by the subcutaneous method. The insoluble material suspended in the vehicle is removed from the cell in any suitable manner.

It will be understood that the usual procedures and precautions followed in conducting electrolytic processes are to be borne in mind in conducting the herein-disclosed process. Since such procedures and steps do not form any part of this invention, however, they are not set forth herein.

The aqueous sodium chloride solution or other electrolyte may vary in concentration over rather wide limits. For example, we may use solutions having an NaCl normality from 0.01 N to 2.0 N. Solutions having a NaCl strength lower than 0.01 N are so high in resistance that the electrolytic process requires an unduly long time from the practical viewpoint; and, furthermore, the resulting complex does not possess properties as desirable as otherwise. Solutions having NaCl concentrations higher than 2.0 N are not recommended as the ACTH is "salted-out". We have obtained excellent results with solutions from 0.10 to 0.15 normality.

The ACTH dissolved in acidified water, as hereinafter indicated, is introduced into the electrolyte. Any commercially- and pharmaceutically-acceptable ACTH may be employed. However, we have found that the purer the ACTH, i.e., more units per milligram, the less zinc compound is needed to effect coprecipitation of most of the ACTH.

We have also found that the ACTH may be employed in widely varying amounts, practical dosage considerations for therapy being the determining factor. For example, while, theoretically, very small or very large quantities of ACTH could be used such would be impractical. Between such extremes it may be said that an amount of

dissolved ACTH sufficient to give compositions of practical utility can be used. We presently prefer to employ an amount of ACTH equivalent in biological activity to from 20 to 80 units per 0.2 to 3.0 mg. of elemental zinc.

If desired, other substances may be added to the electrolyte in accordance with our invention. Such other substances include:

- a. preservatives, e.g., phenol or benzyl alcohol in amounts normally used in pharmaceutical practice; and/or
- b. adjuvants, e.g., glycerol, to achieve isotonicity; and/or
- c. local anaesthetics, e.g., procaine hydrochloride; and/or
- d. other therapeutic substances not incompatible with the other ingredients employed.

As aforesaid, the process of this invention does not require any special electrolytic procedures or precautions. Temperatures below, at, or somewhat above normal room temperature (20°-30°C.) can be used, practical considerations usually dictating the prevailing room temperature as that used. It is important, however, that means for effective agitation of the mixture during the electrolysis should be supplied for best results. Inadequate agitation can cause the formation of substantial amounts of metal compounds in forms other than the desired alpha-zinc hydroxide form or local heating or denaturation of protein and non-uniform incorporation of ACTH in the precipitate.

Insofar as the current density employed in accordance with the present process is concerned, this too can be varied over wide limits. As illustrative, we note that current densities between 0.25 to 2,500 milliamperes per square centimetre give desirable results. We have obtained advantageous results with current densities from 2.5 to 25 milliamperes per square centimetre.

The initial pH of the solution may vary within wide limits. It should not be so low as to permit the zinc anode to be attacked and Zn^{++} to be formed in solution before electrolysis starts as this leads to undesirably high proportions of zinc compound to ACTH in the final complex or precipitate. An initial pH from 2 to 5 has been found advantageous. ACTH is not very stable at a pH over 6.

As the electrolysis proceeds the pH gradually rises. Precipitation starts at a pH of 5-6. The pH continues to rise to 7 to 7.5 and remains thereat for the remainder of the electrolysis. The alpha-zinc hydroxide appears to act as its own buffer to maintain the pH at 7 to 7.6. After electrolysis has been completed, the pH may be increased, if desired, by addition of suitable basic materials.

Where the ACTH is added to the electroly-

tically-prepared zinc compound in accordance with the present process after preparation of said compound, it is understood that the addition should be conducted prior to any significant crystallographic change of the zinc compound. For example, in the case of alpha-zinc hydroxide, such addition should occur within a few minutes after the conclusion of the electrolysis. If an hour or more is allowed to elapse before addition of the ACTH to the zinc precipitate only small amounts of the ACTH are incorporated in the precipitate. In this connection, it is found that these amounts are even less than 25% of the amount of ACTH which is incorporated when the electrolysis is conducted in the presence of the ACTH.

We intend to cover complexes prepared by the aforesaid "in situ" method as well as complexes prepared by adding the ACTH to the electrolytically-prepared zinc compound within a few minutes after preparation of said compound. The term "co-precipitated", as used herein, refers to both of said methods.

As hereinbefore noted, the compositions obtained in accordance with our present invention possess useful and advantageous properties in connection with the treatment of conditions for which ACTH is indicated. In addition, it has surprisingly been found that such compositions possess superior aging properties than even those made in accordance with the aforesaid Specification No. 757,934.

The usual initial dose of the composition of the adrenocorticotropin complex as prepared in the following examples is one cc. daily, injected subcutaneously or intramuscularly. Each cc. contains 40 U.S.P. units. Such dosage may be individualised to the requirements of each patient and the disease under treatment. 20 U.S.P. units may suffice but 80 to 100 units may be required in some cases. The enhanced and prolonged acting composition so prepared allows for lower doses and less frequent administration than that attributed to previously available products. Once symptoms have been controlled, dosage may be diminished in keeping with the requirements of each case and the interval between such doses increased to once every three or four days.

In order more fully to illustrate our invention but without limiting it thereto, we set forth the following examples.

EXAMPLE I

2000 cc. of an aqueous solution was prepared which contained per cc. 10 mg. benzyl alcohol as a preservative, and 6 mg. sodium chloride to render it isotonic. In this solution, corticotropin was dissolved in quantity sufficient to provide 40 U.S.P. units per cc. After adjustment of the pH to 3.0 with hydrochloric acid, the solution was sterilised by filtration through a bacteriological candle.

Using aseptic manipulations, a sterile platinum cathode and a sterile zinc anode were introduced into the above solution, together with a stirring assembly, and an electric current of 475 milliamperes was passed through the solution which was kept well agitated. The formed alpha-zinc hydroxide coprecipitated the corticotropin, and after about 3.5 hours an amount of alpha-zinc hydroxide containing 2 Gm. zinc had been formed which carried with it practically all of the corticotropin. The pH of the suspension was 7.5.

EXAMPLE II

150 cc. of an aqueous solution was prepared which contained per cc. 13.3 mg. benzyl alcohol as a preservative, and 8 mg. sodium chloride to render it isotonic. A platinum cathode and zinc anode were introduced into the above solution, together with a stirrer, and an electric current of 475 milliamperes was passed through the solution which was stirred continuously. After about 25 minutes an amount of alpha-zinc hydroxide was formed which contained 0.2 Gm. zinc. At this point the electrodes were disconnected and 50 cc. of an aqueous solution containing 8000 U.S.P. units of corticotropin, previously adjusted to pH 3.0 with hydrochloric acid, were added to the well stirred suspension. After the adsorption of the corticotropin was practically completed, the pH was readjusted to 7.5 with a sodium hydroxide solution.

EXAMPLE III

200 cc. of an aqueous solution was prepared which contained per cc. 9 mg. sodium chloride. In this solution corticotropin was dissolved in quantity sufficient to provide 40 U.S.P. units per cc. After the adjustment of the pH to 3.0 with hydrochloric acid a platinum cathode and a zinc anode were introduced into the above solution together with a stirrer. An electric current of 450 milliamperes was then passed through the solution which was stirred continuously. The formed alpha-zinc hydroxide coprecipitated the corticotropin, and after about 30 minutes an amount of alpha-zinc hydroxide containing 0.2 Gm. zinc had been formed which carried with it practically all of the corticotropin. The pH of the suspension was 7.55.

EXAMPLE IV

200 cc. of an aqueous solution was prepared which contained per cc. 9 mg. sodium chloride. In this solution corticotropin was dissolved in quantity sufficient to provide 10 U.S.P. units per cc. After the adjustment of the pH to 3.0 with hydrochloric acid a platinum cathode and a zinc anode were introduced into the above solution together with a stirrer. An electric current of 450 milliamperes was then passed through the solution which was stirred continuously. The formed alpha-zinc hydroxide coprecipitated

the corticotropin, and after about 30 minutes an amount of alpha-zinc hydroxide containing 0.2 Gm. zinc had been formed which carried with it practically all of the corticotropin. The pH of the suspension was 7.45.

EXAMPLE V

200 cc. of an aqueous solution was prepared which contained per cc. 9 mg. sodium chloride. In this solution corticotropin was dissolved in quantity sufficient to provide 80 U.S.P. units per cc. After the adjustment of the pH to 3.0 with hydrochloric acid a platinum cathode and a zinc anode were introduced into the above solution together with a stirrer. An electric current of 450 milliamperes was then passed through the solution which was stirred continuously. The formed alpha-zinc hydroxide coprecipitated the corticotropin and after about 30 minutes an amount of alpha-zinc hydroxide, containing 0.2 Gm. zinc, had been formed which carried with it practically all of the corticotropin. The pH of the suspension was 7.6.

EXAMPLE VI

2500 cc. of an aqueous solution was prepared which contained per cc. 6 mg. sodium chloride and 10 mg. benzyl alcohol. In this solution corticotropin was dissolved in quantity sufficient to provide 40 U.S.P. units per cc. After the adjustment of the pH to 3.0 with hydrochloric acid a platinum cathode and a zinc anode were introduced into the above solution together with a stirrer. An electric current of 200 milliamperes was then passed through the solution which was stirred continuously. The formed alpha-zinc hydroxide coprecipitated the corticotropin, and after 3 hours an amount of alpha-zinc hydroxide containing 0.65 gms. zinc was formed which carried with it practically all of the corticotropin. The pH of the suspension was 7.9.

WHAT WE CLAIM IS:

1. Process for making a preparation suitable for injection which comprises forming

a substantially water-insoluble complex of electrolytically-prepared alpha-zinc hydroxide or basic zinc salts and adrenocorticotrophic hormone.

2. Process according to claim 1 wherein the alpha-zinc hydroxide is prepared in the presence of the adrenocorticotrophic hormone.

3. Process according to claim 2 wherein the alpha-zinc hydroxide is prepared in an electrolytic cell having a zinc anode, a platinum cathode and an electrolyte comprising aqueous sodium chloride.

4. Process according to claim 3 wherein the adrenocorticotrophic hormone is present in an amount of 20 to 80 U.S.P. units for each 0.2 mg. to 3.0 mg. of elemental zinc present in the alpha-zinc hydroxide formed.

5. Process according to claim 4 wherein the normality of the sodium chloride is within the range from 0.10 to 0.15 N.

6. Process according to claim 5 wherein the initial pH is from 2 to 5.

7. A composition suitable for injection which comprises a substantially water-insoluble complex of electrolytically-prepared alpha-zinc hydroxide or basic zinc salts and adrenocorticotrophic hormone, said complex being characterised by fine particle size, ease of suspension in suitable vehicles and resistance to breakdown when subjected to alternate freezing and thawing.

8. A composition as claimed in claim 7 wherein the adrenocorticotrophic hormone is present in an amount of 20 to 80 U.S.P. units for each 0.2 mg. to 3.0 mg. of elemental zinc present in the alpha-zinc hydroxide.

9. Processes and compositions substantially as claimed herein.

BROMHEAD & CO.,
Chartered Patent Agents,
St. Paul's Chambers,
19/23, Ludgate Hill,
London, E.C.4.